# EAST Search History

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 22 | piechaczyk.inv. | US-PGPUB; USPAT; EPO; DERWENT | OR | OFF | 2006/08/19 05:10 |
| L2 | 2 | piechaczyk.inv. and idiotype | US-PGPUB; USPAT; EPO; DERWENT | OR | OFF | 2006/08/19 05:16 |
| L3 | 215 | (anti-idiotype and idiotype).clm. | US-PGPUB; USPAT; EPO; DERWENT | OR | OFF | 2006/08/19 05:17 |
| L4 | 4720 | (anti-idiotype and idiotype) | US-PGPUB; USPAT; EPO; DERWENT | OR | OFF | 2006/08/19 05:17 |
| L5 | 1059 | (anti-idiotype and idiotype) and implant | US-PGPUB; USPAT; EPO; DERWENT | OR | OFF | 2006/08/19 05:17 |
| L6 | 58 | (anti-idiotype and idiotype) and ((implant or inplant) with cell) | US-PGPUB; USPAT; EPO; DERWENT | OR | OFF | 2006/08/19 05:18 |

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| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 1 | piechaczyk.inv. and thermodynamic | US-PGPUB; USPAT; EPO; DERWENT | OR | OFF | 2006/08/19 05:31 |
| L2 | 1 | piechaczyk.inv. and thermodynamic and (therapeutic or therapeutically) | US-PGPUB; USPAT; EPO; DERWENT | OR | OFF | 2006/08/19 05:31 |

[0085] The **thermodynamic** and kinetic properties of antibodies produced by these
different cell types determined by surface plasma resonance according to the
BlAcore technique (Pharmacia Biosensor) proved to be identical to those of the
initial Tg10 antibody.

[0086] Retroviral vectors were then used to infect primary mouse skin fibroblasts (human retroviral infection and hepatocytes (transfection). The
antibody production was respectively:

[0087] 10 to 20 ng/10.sup.5 cells/3 days, and

[0088] 1 to 10 ng/10.sup.5 cells /4 days.

[0089] Likewise, the characteristics of the antibodies produced were the same
as those of the initial antibody.

[0090] IV - In Vivo Experiment

[0091] C2C12 cells genetically modified and which retained the capacity to
differentiate into myotubes were implanted via injection in the forelegs of 4
syngenic C3H mice on the basis of 10.sup.7 cells per foreleg.

[0092] In 3 of 4 mice, the production of recombinant antibodies having retained
the **thermodynamic** properties, and the recognition property of the initial
antibody antigen was followed for two months. The quantity of antibody
produced was regularly elevated from the base level to a production of
approximately 100 ng/ml of serum.

[0016] The in vitro production of antibodies, fragments of antibodies or derivatives of antibodies such as chimerical antibodies, by genetic engineering in eucaryotic cells has also already been described, for example, in European patents published under numbers 120 694 and 125 023. The injection into patients of **therapeutic** antibodies aims to target antigens involved in pathology in order to neutralize either directly, or through a chain of metabolic or immune events, one of the causal agents of the disease. Examples of such **therapeutic** strategies include treatment or prevention of B lymphomas (Yefenof, E., Picker, L. I., Scheuermann, R. N., Vitetta E. S., Street, N. E., Tucker, T., Uhr, J. W., Current Opinion in Immunology, 5, 740-744, 1993).

[0017] The international patent application published under No. WO 94/29446 describes the intracellular expression of DNA sequences coding for antibodies. This approach permits considering direct in vivo gene therapy for pathology involving cellular components which are not accessible with traditional vaccination methods or based on the in vivo production of recombinant antigens. The DNA sequences expressed by the genetically modified cells in accordance with the method described in the international patent application WO 94/29446 are therefore essentially characterized by the fact that they include an antibody gene modified so that the antibody is not secreted.

[0018] The present invention aims, on the other hand, to implement the in vivo expression of antibody genes by cells which secrete said antibodies in the blood circulation of the mammal carrying the cells genetically modified by the antibody gene.

[0019] This invention is based on the demonstration that different cell types, other than those naturally producing antibodies are capable, after

genetic
modification, of producing antibodies in a stable fashion in vivo.

[0020] In fact, the plasmocytes, which are cells specialized for antibody
production, are poor candidates for the long-term production of **therapeutic**
antibodies through gene transfer; plasmocytes have a reduced life, on the order
of several days, and the fact that they already produce another antibody is
likely to lead to associations or recombinations between the antibody chains
naturally produced and the antibody expressed by the gene transferred, which is
highly damaging to the **therapeutic** effect sought. It was therefore important
to demonstrate that cell types not specialized for the natural production of
antibodies were capable of accepting a gene transfer, expressing in vivo a
**therapeutic** antibody and secreting beneficially regulated sustained levels of
antibodies in the blood circulation of a mammal.

[0021] As a result, the present invention concerns a biological material for
preparing a pharmaceutical composition for treating a mammal by gene transfer,
comprising,

[0022] either at least a nucleic acid sequence containing a **therapeutic** gene
and in a form enabling in vivo transfer of said gene into the cells of the
mammal,